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THE ANTISCORBUTIC VITAMIN IN BREAD

A Discussion of Guinea-Pig Feeding Experiments
Carried Out by Dr. F. M. Wells and David Chidlow

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A DISCUSSION OF GUINEA-PIG FEEDING EXPERIMENTS CARRIED OUT BY
DR. F. M. WELLS AND DAVID CHIDLOW

In February, 1921, Dr. Wells and the writer initiated a preliminary guinea-pig feeding experiment to determine the degree of resistance to scurvy through the use of different types of bread. This was carried out at the Military Hospital, Ste. Anne's de Bellevue, Quebec, where the duties of Dr. Wells as a medical officer of the Canadian Military Forces required his presence daily.

Dr. Wells's experience at Lister Institute in London had shown that a diet composed of feed oats, wheat bran, and normal cow's milk, sustained guinea-pigs in robust health, reproduction, and growth; but that the same diet with the milk autoclaved for two hours at 150 degrees centigrade, invariably produced death from scurvy within thirty days, and usually, within twenty-two days.

In the following experiments, the Lister basal scorbutic diet was fed to all guinea-pigs, and, in addition, bread as specified. Thirteen healthy guinea-pigs, averaging 420 grams each, were divided into three groups, and fed as follows:

- Group I.—White bread delivered to the hospital for general consumption.
- Group II.—Bread made in laboratory from white flour and mixed germ and scutellum, lard, sugar, salt, yeast, and tap water.
- Group III.—Bread made in laboratory from freshly ground wheat meal, lard, sugar, salt, yeast, and tap water.

RESULTS OF PRELIMINARY FEEDING EXPERIMENT

- Group I.—*White Bread*.—All guinea-pigs died of scurvy within 23 days, and within an average of 21

days. Post mortem examination disclosed: "Marked hemorrhages of femoral muscles, intercostal spaces, and costochondral junctions; tooth pulp destroyed, and the epiphyseal lines showing marked evidence of advanced scurvy."

Group II.—*Germ Bread*.—Feeding was continued for 30 days, the pigs remaining healthy. On the 19th day one of the group gave birth to 3 healthy young. A pig was chloroformed at 30 days, but showed no trace of scurvy.

Group III.—*Wheat Meal Bread*.—Feeding continued up to 30 days; then a pig was chloroformed and post-mortemed. No trace of scurvy was discernible. One pig died of intestinal trouble on the 9th day of the experiment, but no trace of scurvy existed. Two pigs in this group gave birth to healthy young.

The results of this experiment made upon a small number of animals required confirmation with a larger group, and also suggested the possibility of making white bread so that it would protect against scurvy.

The second series was planned to contain 22 animals in each of 6 groups. The composition of the commercial white bread used in Group I of the first Series was not accurately known, although it was known that a yeast substitute was commonly used by the bakery supplying the hospital; so it was decided to make all the breads in the laboratory of James Strachan Limited, of Montreal, who had unusual facilities for carrying this plan into effect.

All breads were made with tap water, salt 1.5, lard 1, sugar 2, and yeast for each 100 parts of flour, with the exceptions noted below for certain of the groups. The patent flour contained 13.5% gluten.

Group 1.—White bread,—5 hour dough using 1.4% yeast.

Group II.—White bread,—1 hour dough using 5% yeast (Yeast X 3.5).

Group III.—White bread,—1 hour dough using 5% yeast and 3% of Canadian-made malt extract containing approximately 30% moisture.

Group IV.—Bread made from mixture of white flour and mixed wheat germ and scutellum. Yeast 5%.

Group V.—Bread made from hard wheat ground into meal in the laboratory the day before use. Yeast 2.3%.

Group VI.—Bread made of wheat germinated for 15 hours, then crushed into a pulpy dough, to which was added salt and 2.3% yeast.

PURPOSE OF FEEDING EXPERIMENT

Group II was designed to determine the degree of protection afforded by additional yeast.

Group III containing extra yeast and malt extract was expected to show some antiscorbutic potency.

Group IV was a repeat of No. II in Series I, which showed a large proportion of Vitamin C—contrary to the report of the British Medical Research Committee, which stated on page 44, that wheat germ contained no antiscorbutic vitamin. The germ used in these experiments was treated to destroy toxicity, remove bitter taste, and still keep the vitamins intact.

Group V was also a confirmatory test of Group III in Series I, and was based on a known deficiency of Vitamin C in wheat meal nine weeks after milling.

Group VI was planned to develop a large degree of antiscorbutic vitamin, partly founded on the practical results achieved by soaking beans in the cure and prevention of scurvy among British troops during the Great War.

In the selection of guinea-pigs for the different groups care was taken to choose some about 400 grams in weight; some young pigs weighing about 250 grams, to determine influence of breads upon growth; and some pregnant animals weighing more than 400 grams, to observe effects of deficiency of Vitamin C in relation to abortion. Dr. Wells and others had noticed that miscarriages and still-born children were often associated with poor teeth in the mothers; which they attributed

largely to incipient scurvy. The pregnant pigs were included to test this hypothesis.

RESULTS OF FEEDING EXPERIMENT SERIES II

- Group I.—*White Bread*.—Of the 22 guinea-pigs, one died in 10 days of an undetermined cause, but not scurvy. Fifteen died of scurvy within an average of 21 days, the last one at 24 days; the pregnant pigs died of scurvy within 18 days, one in 17 days, and one in 19 days. Post mortem examinations revealed definite characteristic abnormalities in the tissues. There were very marked hemorrhages in the femoral muscles, enlarged and hemorrhagic costochondral junctions, and the livers were clay-colored with lighter spots. All 21 pigs were emaciated. The teeth of all the pigs were very loose, the pulp was destroyed, and the epiphyseal lines showed marked evidence of advanced forms of scurvy. Microscopic examination of tooth pulp revealed no remaining trace of cellular organization, no trace of cell nuclei, nor any trace of interstitial cement substance. Microscopic examination of embryos of pregnant pigs showed them to be hemorrhagic, and further examination of sections of embryos showed that the blood vessels and cellular tissue had undergone the process of fibrification and were no longer recognizable.
- Group II.—*White Bread (Yeast X 3.5)*.—One of this group died of intestinal trouble on the 12th day. One pregnant pig aborted on the 9th day and died on the 12th. Another pregnant pig died of scurvy on the 18th day, having hemorrhaged embryo. The other 19 died of advanced scurvy with general condition as de-

scribed in Group I above. The average time of resistance was 25.5 days—the last one dying on the 28th day.

Group III.—*White Bread With Extra Yeast and Malt Extract*.—Nine pigs in this group died of intestinal disturbances within 12 days, showing no symptoms of scurvy. The other 13 pigs died of scurvy with physical conditions as in Group I. The average period of resistance was 21 days—one dying in 15 days, and one in 26 days, the bones of the latter being very brittle.

Group IV.—*Germ Bread*.—The feeding experiment with this group was ended on the 37th day, because none of the animals died of scurvy. Some of the group died of intestinal trouble up to the 21st day of the feeding period, but they showed no scorbutic symptoms. Two pigs were chloroformed on the 37th day and the post-mortems disclosed total absence of scurvy, teeth sound, internal organs clean and healthy in color and odor, and the animals fat.

Group V.—*Wheat Meal Bread*.—Two deaths occurred in this group on the 24th day, but were not of scorbutic origin. On the 37th day the other 20 were in sound health. Two were chloroformed and carefully examined for scurvy, but were found fat and healthy.

Group VI.—*Germinated Wheat Bread*.—This group and Group IV ate the largest amount of bread. On the 15th day of the experiment some eye trouble developed followed by intestinal disturbance. In 3 days following several more pigs developed the same symptoms. The eye trouble suggested xerthalmia, though later this diagnosis was rejected. Since it seemed impossible to locate the cause of the difficulty, the feeding of this group was discontinued.

However, up to the last (18th) day of the experiment, no deaths due to scurvy had occurred. All teeth were sound.

DISCUSSION AND CONCLUSIONS

The drain on the Vitamin C reserve is vividly shown in the pregnant animals of Group I. The average resistance of the non-pregnant portion was 21 days; that of the pregnant portion, 18 days; the pregnant pigs dying 13% earlier than other pigs on the same diet.

The large excess of yeast in Group II increased resistance to scurvy uniformly about 22%. This result cannot be due to protection of the Vitamin C reserve through the addition of Vitamin B, because there was already a large amount of Vitamin B present in the wheat bran, oats, milk, and initial unit of yeast in the bread of Group I, Series II. Therefore the additional yeast must contain a degree of the antiscorbutic vitamin sufficient to prolong life 22% longer than a scorbutic diet with less yeast.

It was disappointing and surprising to find that malt extract and excess yeast gave less protection than excess yeast alone. There was good foundation for the belief that malt extract in sufficient quantity would afford protection against scurvy. First, the resting (ungerminated) grain freshly ground afforded adequate protection, as shown in Group III, Series I. Secondly, it has been shown that germinated pulses and grains developed more protection against scurvy than the resting seeds. It was therefore reasonable to expect that an aqueous extract of germinated grain would contain all of the water soluble vitamins, including Vitamin C. A malt extract of a light color, having a diastasic value of 180 Lintner, was chosen and designed for use in the experimental bread; but by some crass mistake, in the laboratory another malt extract, made during the war, of mixed grain and malt, and containing little or no diastase, was used in place of the one designed. This explanation is necessary in order that the results here reported shall not prejudice further trials. There is the soundest ground for believing that ulti-

mately, at all events, properly made and evaporated extracts will furnish adequate protection against scurvy when used in white bread.

The outstanding facts are: That ordinary white bread fed to guinea-pigs in addition to wheat bran, whole oats, and whole cow's milk autoclaved for 2 hours at 150 degrees Centigrade, causes death from scurvy within 24 days; while the same flour and ingredients in the bread, supplemented by wheat germ incorporated during breadmaking, with the same basal ration, entirely protects against scurvy. Freshly ground wheat meal also protects against scurvy, in face of the negative report of the British Medical Research Committee concerning wheat meal. However, it is generally admitted that oxidation lowers the antiscorbutic value of wheat meal rapidly. Of one thing we are certain,—freshly ground wheat meal is an efficient antiscorbutic.

A further conclusion is that white bread fed with a scorbutic basal ration affects pregnant guinea-pigs more quickly and seriously than non-pregnant ones, causing abortion and still-born young; while the presence of wheat germ and scutellum with white flour, or freshly ground wheat meal, protect against scorbutic conditions.

Considering that Dr. Wells reports that 97% of the population of the Province of Quebec have decayed teeth, the fact that the collapse and destruction of tooth pulp is the first observable symptom of scurvy is of momentous interest. In connection with some unpublished experiments on guinea-pigs, Dr. Wells reports that hemorrhage of the odontoblastic cells (tooth pulp) begins as early as the 9th day of the feeding experiment. When death occurs in 17 days—as in Group I, Series II—with teeth loose and hemorrhaged embryo, we can easily realize that destruction of tooth pulp must begin at a very early period.

It is rather important to note that yeast contains the antiscorbutic Vitamin C—although in small amount, because increasing the yeast 3.5 times provides 22% more protection against scurvy than ordinary bread, which in the case of this experiment had double the quantity of yeast commonly used by bakers, many of whom replace half the yeast by a substitute, thereby using about 0.70% yeast.

Since the guinea-pigs had wheat bran before them all the time, these experiments show that bran contains no appreciable amount of the antiscorbutic vitamin, and therefore its inclusion in the form of an addition to white flour bread could not serve as a source of Vitamin C, though it might be considered valuable as roughage.

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